

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Validation of Growth Differentiation Factor (GDF-15) as a Radiation Response Gene and Radiosensitizing Target in Mammary Adenocarcinoma Model

Hargita Hegyesi<sup>1</sup>, James R. Lambert<sup>2</sup>, Nikolett Sándor<sup>1</sup>,  
Boglárka Schilling-Tóth<sup>1</sup> and Géza Sáfrány<sup>1</sup>

<sup>1</sup>*Frédéric Joliot-Curie National Research Institute for Radiobiology and Radiohygiene,*

<sup>2</sup>*Department of Pathology, University of Colorado,  
Anschutz Medical Campus, Aurora, CO,*

<sup>1</sup>*Hungary*

<sup>2</sup>*USA*

## 1. Introduction

In this chapter we summarize the role of cytokines in the radiation response, focusing in particular on growth differentiation factor-15 (GDF-15), and give a brief overview of our ongoing experiments in a mouse mammary carcinoma model.

The principal function, receptor, and signaling pathway of GDF-15 remain uncertain, although several of its biological activities have already been described; the exact role of GDF-15 in cancer progression also remains poorly understood. Increased GDF-15 expression is a common feature of many cancers. Several studies have observed upregulation of GDF-15 mRNA and protein in tumor biopsy. Serum GDF-15 levels are often markedly elevated in cases of metastatic cancer, and appear to occur in parallel with the stages and extent of disease, particularly in cases of prostate and melanoma [Senapati et al., 2010, Boyle et al., 2009]. Indeed, a number of studies have described an antitumorigenic function for GDF-15, by which it induces apoptosis and may negatively affect tumor growth [Cekanova et al., 2009, Jutooru et al., 2009].

Prevention by eliminating tumor promoters, early diagnosis and new target treatment are keys to reduce the numbers of deaths caused by breast cancer. In our present study, we propose that GDF-15 increases radioresistance. We demonstrate that down regulated mouse GDF-15 by RNA interference improves the radiosensitivity of the tumor in an LM2 mouse breast cancer model. In this regard, GDF-15 overexpression in breast cancer cell has been shown to supply important cytoprotective roles and resistance to radiation treatments. The paradoxical role of GDF-15 in breast cancer could be related to its pleiotropic effect on different signal pathway.

Recent research has focused on molecular targets for radiation sensitization of cancer cells. It is of particular interest that several potential target proteins involved in radiation sensitization, which have been identified in growth related cellular signaling pathways.

## 2. Radiation response genes in breast cancer cells

Radiation causes DNA damage, which activates ATM (ataxia telangiectasia mutated/ATR (ATM and Rad3-related) protein that in turn promotes activation of receptors/ intracellular signaling pathways and stimulates cell cycle checkpoints, p53 (tumor protein 53) activity, and DNA repair pathways. Radiation generates ionizing events in water in the cytosol that are amplified, thought to be mediated by mitochondria, which generate large amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that inhibit protein tyrosine phosphatase (PTPase) activities. In addition, radiation activates acidic sphingomyelinase and increases the production of ceramide. Inhibition of PTPases leads to a general derepression (activation) of receptor and nonreceptor tyrosine kinases and the activation of downstream signal transduction pathways. Radiation-induced ceramide has been shown to promote membrane-associated receptor activation by facilitating the clustering of receptors within lipid rafts [Balaban et al., 1996, Goldkorn et al., 1997].

The inducible alteration in gene expression is a fundamental molecular event of mammalian cells in response to ionizing radiation, and the fate of cells will at least partially depend upon the inducible changes in expression of some genes involved in these complex regulatory pathways resulting in cell cycle delays, cell killing or apoptosis and DNA repair [Chaudhry et al., 2003, Cucinotta et al., 2002 Vallat et al., 2003]. The cDNA microarray technology, allowing a large-scale expression profiling analysis, can provide tremendous information for elucidating the complex cellular response to radiation. Until now, a great number of radiation-inducible target genes have already been identified. Some of the inducible genes were identified in cells exposed to high and even supra-lethal doses of ionizing radiation (IR) [Roy et al., 2001]; low doses or low dose rates [Ding et al., 2005, Amundson et al., 2003, Mercier et al., 2004]. These reports provide very valuable information for understanding the precise mechanisms of the diverse effects of IR. A simultaneously comparative analysis of gene responses to radiation from low, medium to high doses should be more informative for identifying the inducible genes with a dose-dependent expression.

It is well known that the p53 tumor suppressor plays a key role in mediating apoptosis and the cell cycle checkpoint by various intracellular and extracellular signals including IR. A number of proteins have been identified to be involved in the p53 pathway, including Proliferating cell nuclear antigen (PCNA), Cyclin-dependent kinase inhibitor 1A (CDKN1A/p21), Murine double minute 2 (MDM2), GDF-15, Tumor necrosis factor receptor superfamily member 10B (TNFRSF10B/TRAIL-R2), Tumor protein p53 inducible protein 3 (TP53I3/PIG3) and Growth arrest and DNA damage (GADD45) [Polyak et al., 1997, Contente et al., 2002]. In a microarray investigation showed that, only five genes were significantly induced by the radiotherapy in breast cancer tissue. The genes are DNA damage-binding protein 2 (DDB2), CDKN1A, GDF-15, Glutathione peroxidase 1 (GPX1) and Polo-like kinase 3 (PLK3) [Helland et al., 2006]. Additional studies demonstrated that the levels of mRNA and protein expression of PCNA, c-fos, c-Jun N-terminal kinase 2 (JNK2) and Fos-related antigen 1 (Fra-1) were increased in the mammary epithelial cell line compared to the levels in non-tumorigenic control cells. The transforming factor Rho A was significantly increased only in the tumor cell line. Furthermore, the levels of mRNA and protein expression of Human epidermal growth factor receptor 2 (ErbB2) were significantly increased in the transformed cell line and in tumor cells derived from the transformed cells after injecting them into nude mice [Yun et al., 2010]. A decrease in RbA/p48 protein

expression and mRNA levels was observed in cells treated with double doses of alpha particle radiation in the presence of estrogen, regardless of tumorigenicity. Furthermore, radiation increased c-myc, c-jun and c-fos protein expression in the c-Ha-ras- relative to non-irradiated control cell line [Calaf & Hei, 2004].

The role that cytokines play in radiation toxicity was first discovered and described by Rubin and colleagues [Rubin et al, 1986]. Shortly thereafter, transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) was identified in the circulation of breast cancer patients who developed pulmonary and hepatic complications from chemotherapy for bone marrow transplantation [Anscher et al, 1993]. Chen and colleagues found that subjects undergoing thoracic radiation who had elevated interleukine-1 (IL-1) or IL-6 before or during radiation all developed some degree of radiation changes (clinical or radiographic). Angiogenic factors have also been associated with late pulmonary toxicity. For example, Fibroblast growth factor 2 (FGF2) was markedly elevated in the circulation of most subjects with severe late radiation fibrovascular toxicity of the extremities [Chen et al, 2002].

Presently, we do not yet have adequate markers for the vast majority of clinical needs, and their discovery remains a very high priority in radiation research. The need for these markers has intensified due to the growing number of cancer survivors at risk for developing toxicity from radiation, chemotherapy, surgery, and combinations of all three.

## 2.1 GDF-15 expression in different normal and malignant cells

GDF-15 is expressed at high levels in placenta, macrophages, and epithelial cells. Its expression is very low in tumor cell lines originating from breast, cervix, and lung [Li et al., 2000], but elevated in some metastatic colon and gastric cancers [Buckhaults et al., 2001, Lee et al., 2003]. GDF-15 expression is rapidly induced by a variety of cellular stresses in a p53-dependent and -independent manner, and it mediates cell cycle arrest and apoptosis in response to DNA damage, toxins, anoxia, liver injury, and other cellular stresses [Hsiao et al., 2000, Albertoni et al., 2002, Wilson et al., 2003]. GDF-15 induction may occur via Heat-shock protein 70-2 (Hsp70-2) depletion. In HeLa cells Hsp70-2 depletion resulted in increased p53 protein levels and activity as measured by a reporter gene assay [Rohde et al., 2005]. GDF-15 is a secreted protein that can inhibit tumor cell growth both in an autocrine and paracrine fashion [Tan et al., 2000]. Whereas the antiproliferative effect of GDF-15 depends on the intact TGF- $\beta$  signaling pathway, receptor and mothers against 32 decapentaplegic homolog 4 (Smad4), endogenously expressed GDF-15 is highly cytotoxic also in Smad4-null breast cancer cells [Li et al., 2005, Tan et al., 2000]. Thus, therapeutic strategies targeting GDF-15 are likely to affect also cancer cells with defective TGF- $\beta$  signaling and additionally to induce a so-called bystander effect in GDF-15-responsive tumors.

GDF-15 has been reported to be regulated by many chemicals at the transcriptional level [Li et al., 2000, Baek et al., 2001, Baek et al., 2004, Baek et al., 2005] and some of which are dependent on de novo protein synthesis [Newman et al., 2003]. Transcriptional regulation of GDF-15 is complex, and the promoter sequence has many different cis- and trans-acting promoter elements [Baek et al., 2001]. Induction of GDF-15 expression by doxorubicin, hypoxia and the hypoxia mimetic, cobalt chloride in LNCaP prostate tumor cells strictly was dependent on functional p53. LNCaP cells expressing dominant negative p53 failed to induce GDF-15 mRNA and protein under all of these experimental conditions. Similarly, p53 function was also required for induction of GDF-15 expression in response to high cell

density [Kelly et al., 2009]. In addition, GDF-15 seems to be a potentially important downstream target of three tumor suppressor genes. GDF-15 expression is regulated by the tumor suppressor genes p53 and early growth response (EGR) gene-1 [Baek et al., 2004]. Recently, was identified GDF-15 as a novel negative downstream target of the phosphatidylinositol 3-kinase/protein kinase B (AKT)/glycogen synthase kinase (GSK)-3 $\beta$  pathway [Yamaguchi et al., 2004].

The role of GDF-15 has been implicated directly with cancer, in which both antiapoptotic and proapoptotic effects have been described in a variety of tumor cell types. GDF-15 induction by vitamin D via p53-dependent mechanism and inhibition of prostate cancer cell growth was reported previously [Lambert et al., 2006]. Experimental studies using human prostate LNCaP cells supported the role of GDF-15 in regulation of cell proliferation. Overexpression of GDF-15 by transfection in LNCaP-C33 cells induced aggressive cell growth, whereas knocking down GDF-15 in LNCaP-derived subclones (C81 and LNCaP-Ln3: LNCaP cells highly metastasis to lymph nodes) using antisense oligonucleotides inhibited cell growth and proliferation [Chen et al., 2007]. Also, GDF-15 overexpression in colon cancer cell lines reduced the growth of xenograft tumors [Baek et al., 2001], but serum GDF-15 levels were positively correlated with tumor stage and metastasis [Brown et al., 2003]. Despite ambiguous observations in various tumor cell types, data obtained from clinical studies has established that serum GDF-15 was the best diagnostic marker of bone metastasis in prostate cancer [Selander et al., 2007]. However in the tissue microenvironment various components individually and/or collectively cause tissue damage or injury, leading to inflammation. Inflammatory products, including GDF-15, could contribute to the tumor promotion environment. The tumorigenic function of GDF-15 could be modified by educating the macrophages. Or another possibility is that, thus GDF-15 is secreted from tumor cells together with vascular endothelial growth factor (VEGF) to promote vascular development mediated by serine/threonine-protein kinase B-Raf signaling as reported in malignant melanoma [Huh et al., 2010,].

Individual cancers have different levels of secreted GDF-15 protein because of differences in expression level and variation in the processing of mature GDF-15 [Bauskin et al., 2005]. Serum analyses of cancer patients showed a significant correlation between increased GDF-15 protein levels and the metastatic progression of colorectal, breast, and prostate cancers [Welsh et al., 2003, Koopmann et al., 2004, Baek et al., 2009]. The association of cancer progression with GDF-15 expression resembles that of TGF- $\beta$ , which plays a role as a tumor suppressor during early stages of cancer and as a growth enhancer in later stages [Dumont & Artega 2003].

GDF-15 also induced the transactivation of HER2/neu (ErbB2 tyrosine kinase) in human breast and gastric cancer cells, and this activation stimulated Hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ) protein accumulation and the expression of its target gene via the PI3K/Akt/mTOR and ERK-1/2 signaling pathways. These novel observations provide additional support for the notion that GDF-15 may operate as a positive regulator of tumor progression in certain ErbB2-overexpressing tumors, including breast and gastric cancers [Kim et al., 2008, Klos et al., 2006].

The increase of GDF-15 expression induced via environmental stimuli (irradiation, hypoxia, free radicals etc.) is indicated. Furthermore, several transcriptional factors have been shown to increase GDF-15 expression level in p53 -dependent or independent manner in cancer cells in vitro and in mice in vivo. Potentially GDF-15 is a downstream mediator of the radioprotection of LM2 tumor cells to DNA damage and oxidative stress. This suggests



GDF-15 has significant paracrine effects, which modulate the tumor environment; however what is clear, is that there is a strong evidence for GDF-15 measurement in blood and tissues to detect and monitor cancer progression.

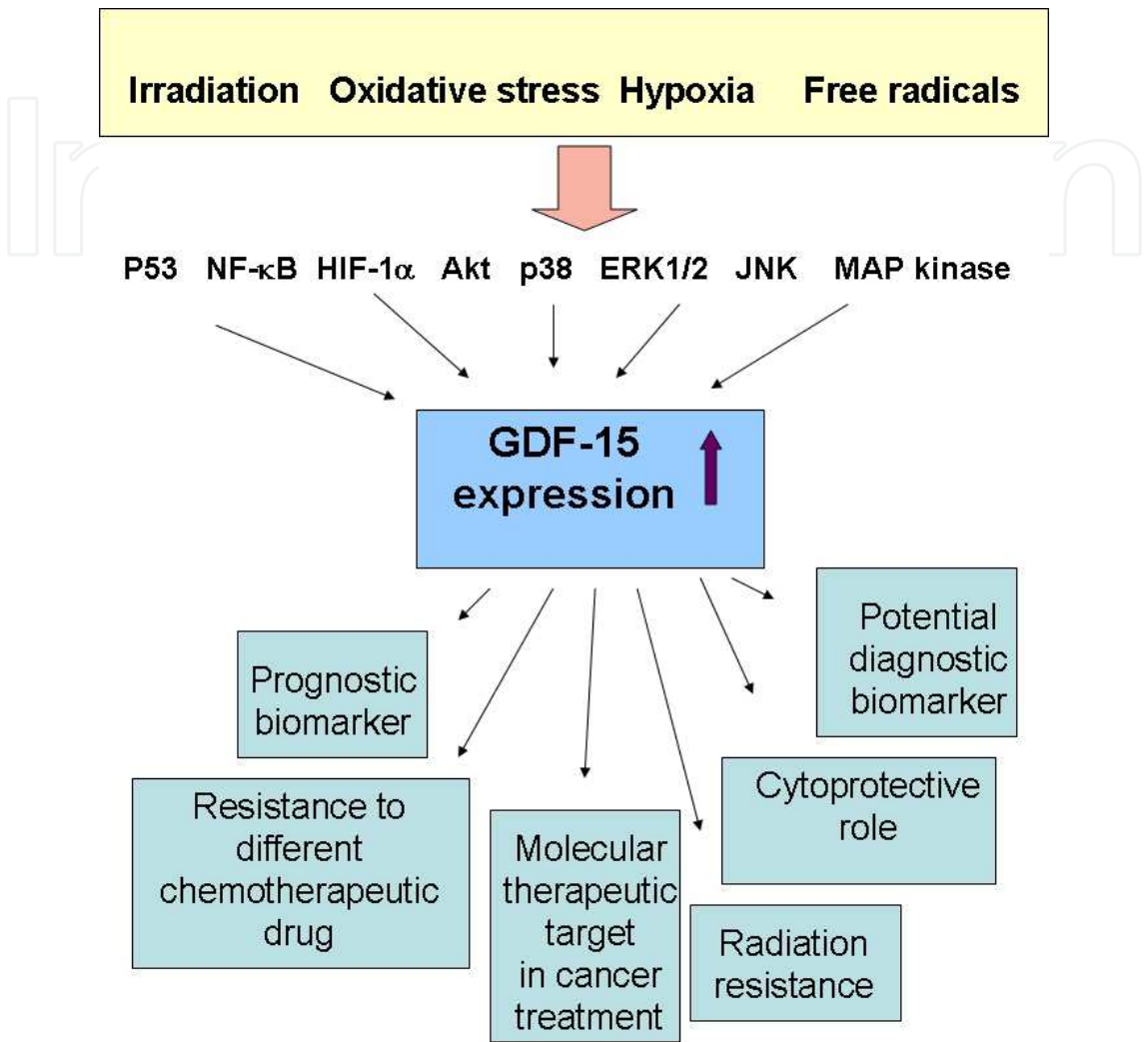


Fig. 1. Pleiotropic effect of GDF-15 overexpression.

3. Research result

3.1 Characteristics of LM2 cells as a model of breast cancer

Several years ago a mammary tumor cell line, LM2, derived from M2 mammary adenocarcinoma which spontaneously appeared in a Balb/c female mouse was described [Galli et al., 2000]. The LM2 cell line has been maintained in culture and grows as poorly differentiated elongated cells. Ultrastructural and immunocytochemistry analysis revealed characteristic features of adenocarcinoma. Cytogenetic studies showed that LM2 cells are fundamentally hypotetraploid. They express metalloproteinases (MMP) and show high levels of plasminogen activator type urokinase (uPA). They were sensitive to nitric oxide (NO)-mediated cytotoxicity when NO derived from an exogenous donor. In vivo, although LM2 cells were able to grow in the lungs, they could not metastasize to the same target organ from s.c. primary tumors. The LM2 mouse mammary adenocarcinoma cell line is a

suitable model to examine different aspects of tumor biology [Hegyesi et al., 2007], in particular those related to the different pathways involved in the metastatic cascade and in the cytotoxicity mediated by NO. LM2 cells, kindly provided from the laboratory of Dr. Lucas Colombo (Research Area, Institute of Oncology Angel H. Roffo, C1417DTB, Buenos Aires, Argentina), were grown in DMEM medium supplied with 1% L-Glutamine and Penicillin/ Streptomycin and 10% FBS.

3.2 Radiation induced gene expression in LM2

It has been reported that GDF-15 is widely expressed, at low levels in different epithelial cells, but its expression is dramatically increased following inflammation, injury, or malignancy [Bauskin et al., 2006]. Our qRT-PCR data showed elevated transcriptional response of GDF-15 in LM2 cells, irradiated with 2 Gy and analyzed 2 hour later, but expression of TGF-β did not show the similar expression changes in these cells. It is likely that LM2 cells differently express TGF-β and the GDF-15.

The 2 Gy-induced changes in the transcript level of the GDF-15 and TGF-β gene were measured by qRT-PCR. The observed changes are shown in Figure 2. GDF-15 mRNA levels increased 2 hours after exposure with 2 Gy ( $p<0.011$  with one-way ANOVA). Expression of TGF-β mRNA decreased 2 hours after exposure with 2 Gy ( $p<0.039$  with one-way ANOVA). The dose-dependency of radiation-induced expression of GDF-15 at 2 hours with γ-ray exposure with doses 0.1 Gy, 2 Gy and 4 Gy was  $196.9\% \pm 17$ ,  $201.01\% \pm 25$  and  $585.63\% \pm 81$ , respectively in LM2 cells.

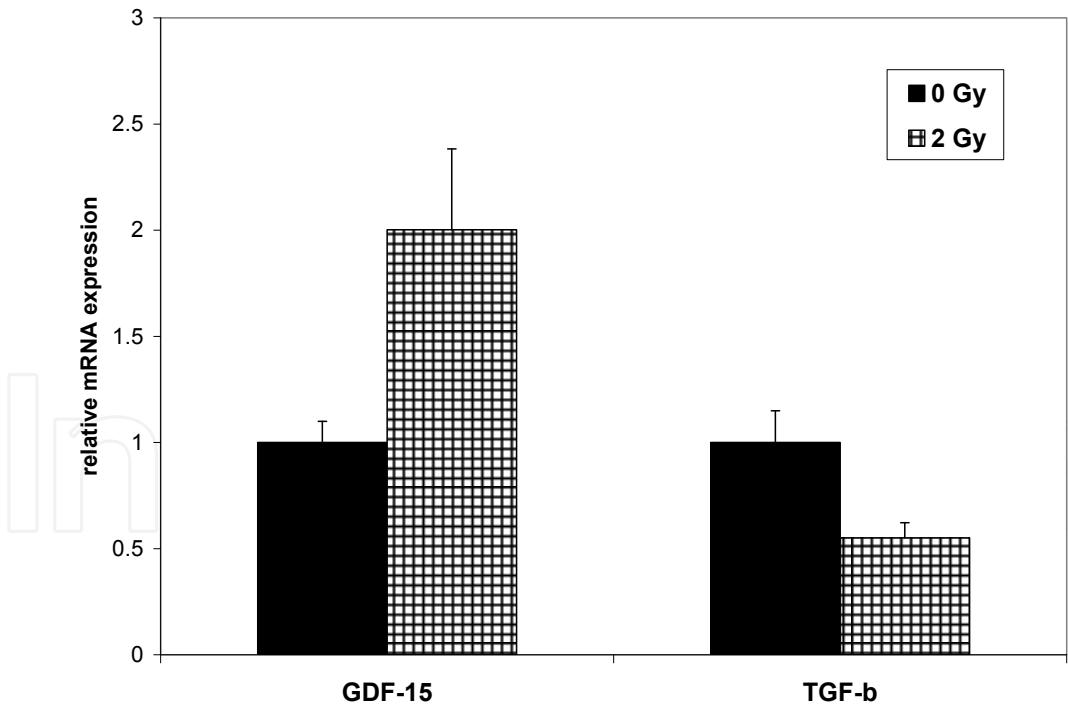


Fig. 2. 2 Gy induced expressional changes of GDF-15 and TGF-β measured by real time PCR. Real-time PCR was performed to test for radiation induced changes in GDF-15 and TGF-β mRNA expression. LM2 cells showed significant increased GDF-15 and decreased TGF-β mRNA expression after irradiation at 2h. The mRNA expression is normalized to the unirradiated controls. Error bars represent standard errors. Measurements were performed in duplicate, and experiments were repeated three times.

The identification of radiation-inducible genes, especially those exhibiting a dose-dependent response, not only expands our knowledge of the mechanisms underlying the diverse biological effects induced by ionizing radiation, but provides candidates for developing novel biomarkers of radiation injury.

3.3 Development of GDF-15 knocked down LM2 stable cell line

To establish a suitable cellular system to investigate a possible role of GDF-15 in breast cancer progression, we examined effect of GDF-15 silencing in LM2 cell lines. To create GDF-15 expression silenced cell lines, we used plasmid vector construct kindly provided by Dr. Lambert (Anschutz Medical Campus, UCD-School of Medicine, Aurora, CO, USA). According to the manufacturer’s protocol of OriGene shRNA (OriGene, Rockville, MD, USA) with different gene-specific shRNA expression pRS vectors (mouse GDF-15 shRNA) transfected into subconfluent LM2 cells for 48 hours. After transfection, puromycin (15 µg/ml) selection was commenced and maintained for two weeks to obtain the puromycin-resistant clones. Four clones were analyzed. The expectation was that a range of knockdown efficiencies will be achieved with at least 1 to 2 clones resulting in very high levels of knockdown. Expression of GDF-15 mRNA was measured by qRT-PCR. (We named the stable shGDF-15 expressing LM2 cells as “shGDF-15#1; #2; #3 or #4”.) The puromycin-resistant stable transfected LM2 cells were propagated in the continual presence of puromycin (15 µg/ml). Total RNA was isolated and used as a template to quantify the level of suppression of GDF-15 by qRT-PCR. GDF-15 expression data from several transfected clones is shown in Figure 3. Results showed that GDF-15 expression was indeed almost completely knocked down in

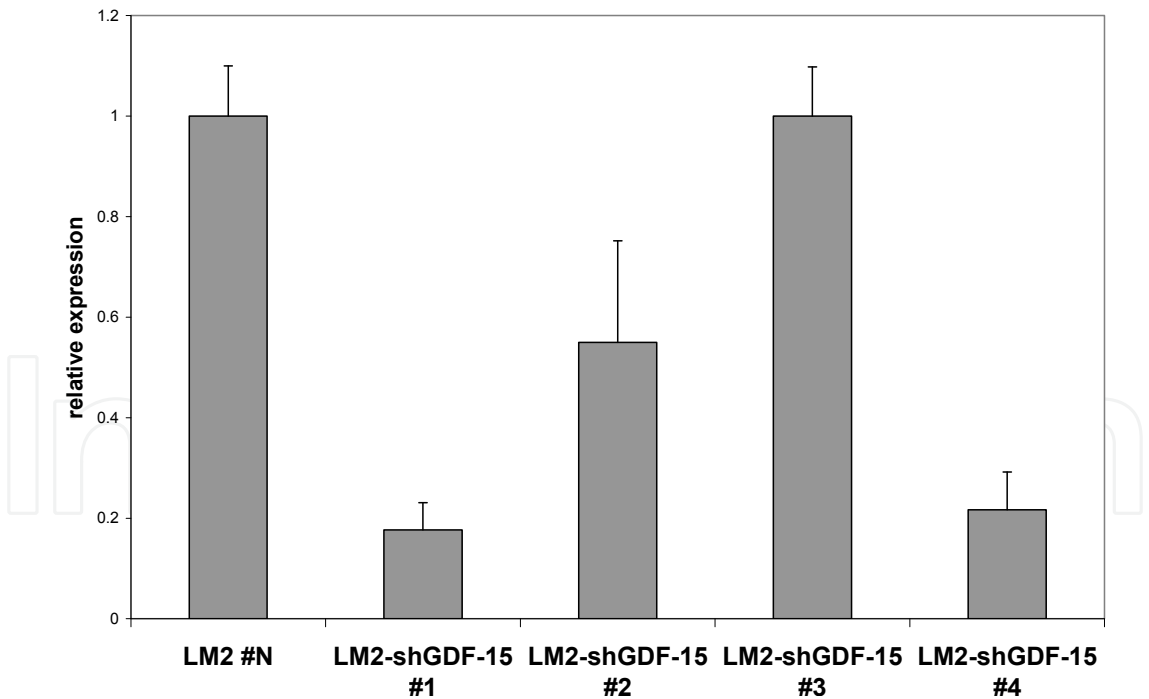


Fig. 3. The efficiency of down regulation of the GDF-15 gene was determined by qRT-PCR. the transfected cells shGDF-15#1 and shGDF-15#4 at mRNA levels, whereas no effect was observed in scrambled shRNA-transfected control cells (LM2 #N). It can be seen that the expression of GDF-15 was most suppressed in the cell clone shGDF-15#1, which was generated from cells transfected with shRNA vector GDF-15#1. Therefore, the clone LM2-



shGDF-15#1 was used for further investigation of the involvement of GDF-15 in the cellular response to radiation.

The effect of 2 Gy exposure on expression of GDF-15 was almost completely abolished in GDF-15 silenced LM2 when compared with LM2#N + 2 Gy IR, as see on Figure 4 (2Gy induced elevation of GDF-15 in LM2#N: GDF-15 201%, LM2-shGDF-15#1: GDF-15 119,31%, was measured, respectively  $p < 0.05$ ).

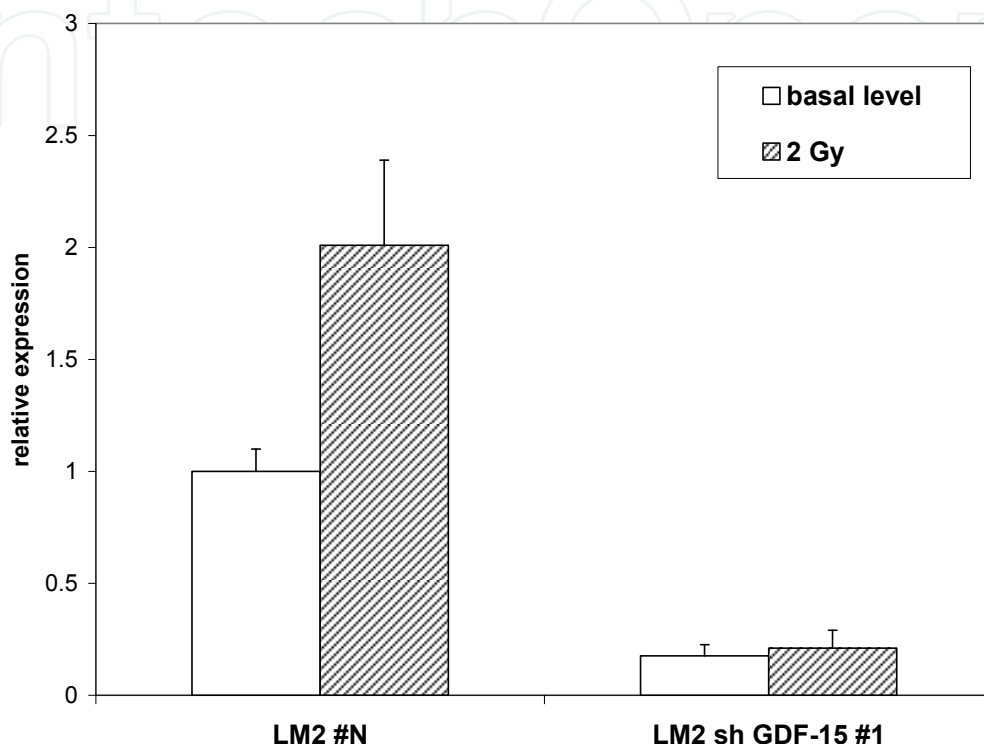


Fig. 4. 2 Gy induced expressional changes of GDF-15 measured by real time qRT-PCR.

### 3.4 Effect of GDF-15 silencing on survival of LM2 cells

In human colorectal HCT-116 cells, knockdown of GDF-15 impairs cell growth and survival [Baek et al, 2001]. Overexpression of GDF-15 in sense GDF-15 cells enhanced basal and indomethacin stimulated apoptosis, whereas the antisense GDF-15 exhibited an attenuated response to indomethacin treatment. Knockdown of GDF-15 in nasopharyngeal NPC cells resulted in growth delay and a reduction of clonogenic survival in response to radiation [Chang et al, 2007].

To gain a better understanding of how GDF-15 is involved in radiation response in mammary carcinoma cells, we determined the effects of stable knockdown of GDF-15 on radioresistance through expression of shRNA targeting GDF-15. In our study clonogenic survival assays further demonstrated that suppression of GDF-15 increased the sensitivity of LM2 shGDF-15#1 cells to radiation doses of up to 2 Gy (Figure 5). Our results suggest that cells with reduced GDF-15 expression were sensitive to radiation-induced cell death (Figure 5,  $p < 0.05$ , LM2#N vs. LM2-shGDF-15#1). For statistical analysis Pearson's t test were used. Differences were accepted as statistically significant if  $p < 0.05$ . These results further validate the specific role of GDF-15 pathway in regulation of radioresistance, and suggest that high level of expression of GDF-15 may be required for radioresistance of breast cancer cells.

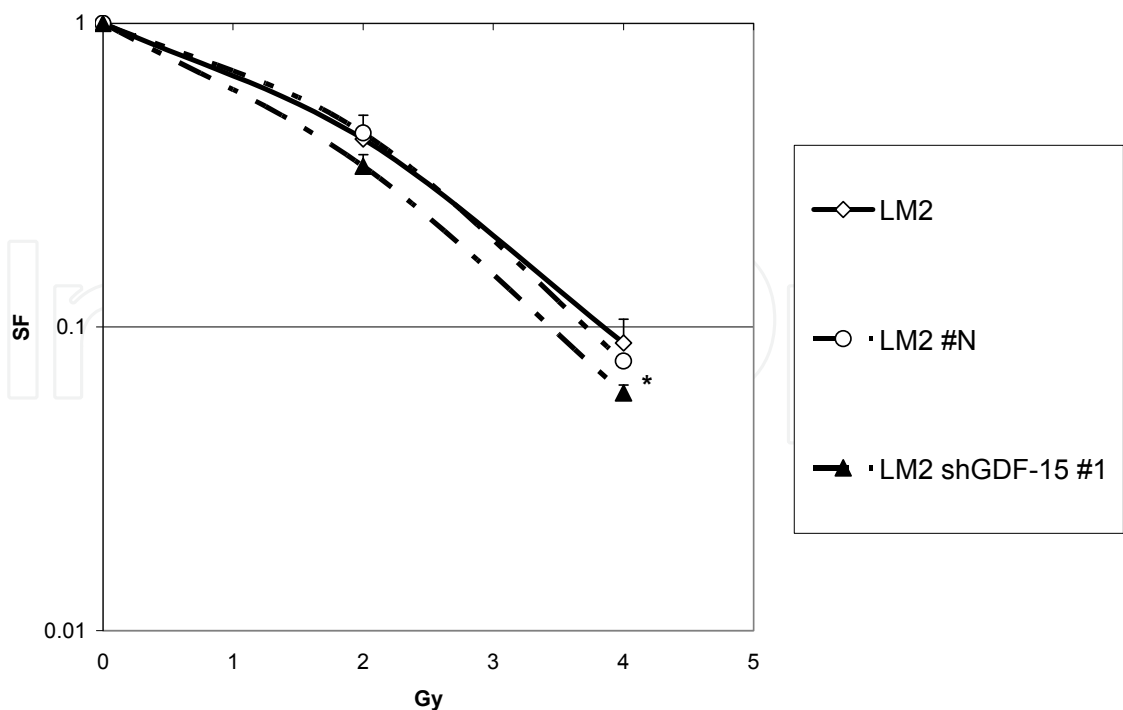


Fig. 5. Clonogenic survivals of irradiated LM2 cells after stable shGDF-15#1 transfection. Clonogenic survival was measured after stable transfection with GDF-15-specific shRNA (shGDF-15#1) in combination with irradiation at 2 Gy and 4 Gy. To examine the additional effects of irradiation all values of clonogenic survival at 0 Gy were set arbitrarily at 1. GDF-15 depleted cells showed significant increased radiosensitivity, after irradiation with 2 Gy or 4 Gy, respectively. Data represent the average values ( $\pm$  SE) of at least three independent experiments. (\*  $p < 0.05$ ).

3.5 Effect of GDF-15 silencing on in vivo tumor growth

The LM2 mammary adenocarcinoma and Balb/c mice was used for tumor growth delay assay. The mice were all female and 10 weeks old at the start of treatment. Exponentially growing cells cultured in DMEM medium supplemented with 10% fetal bovine serum were washed in PBS buffer and inoculated subcutaneously into the right flank. The growth delay assay was performed when the LM2 tumor reached 2 mm in average diameter, (6 days after inoculation).

In order to investigate the relevance of GDF-15 expression in tumor development, we examined whether GDF-15 silencing by shGDF-15 affects tumor growth in vivo. We used stable transfected LM2 shGDF-15#1 cell line or empty vector control LM2#N cells. The proliferation rate of LM2#N, and LM2-shGDF-15#1 cells, in vitro, was not affected significantly by GDF-15 expression and was comparable to the proliferation rate of the parental control cell line (Figure 6a).

We then analyzed whether GDF-15 silencing could affect the tumor growth capacity of these cells in vivo. The GDF-15 silencing clones LM2-shGDF-15#1 and the control LM2#N were injected subcutaneously into the flanks of the Balb/C mice. Tumor growth was observed and measured over a time period of 4 weeks (Figure 6b). Growth was delayed in GDF-15 knockdown cells, while the cells of the control pool retained high proliferative properties. Since the proliferation rate of GDF-15 silencing cells in vitro was almost similar to the

proliferation rate of the control cells, inhibition of tumorigenicity by silencing of GDF-15 observed in vivo might involve in part a paracrine effect of GDF-15 on the host cells.

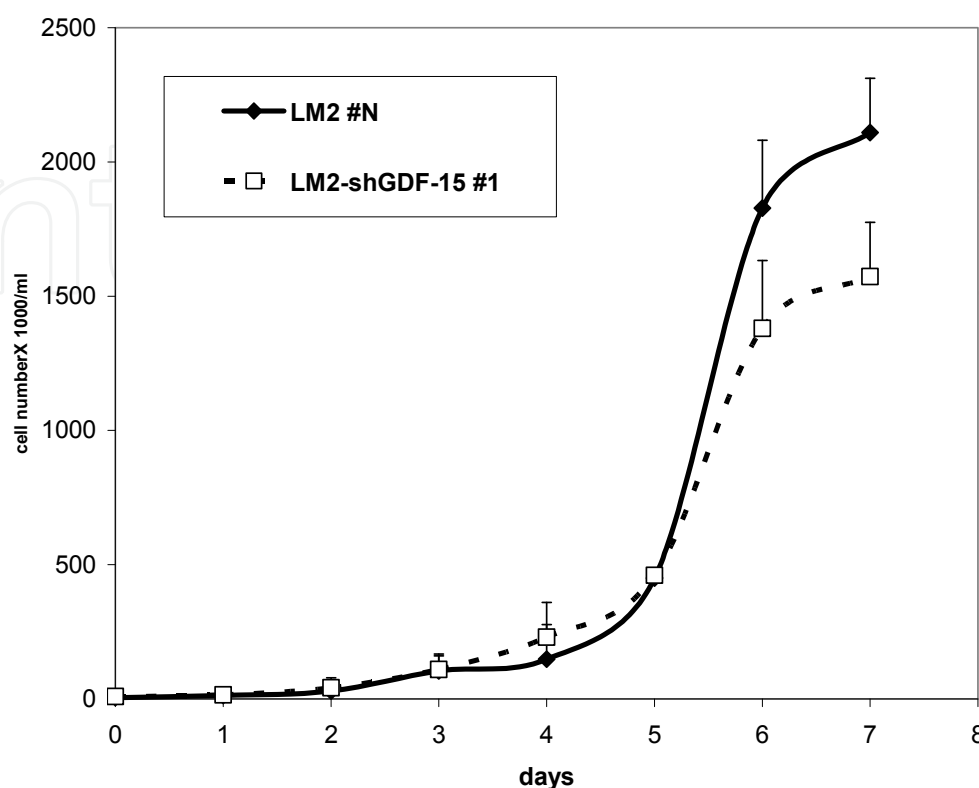


Fig. 6. a Effect of GDF-15 expression on cell growth in vitro.

Cells numbers were determined by manual counting using hemocytometer. Data are expressed as a mean  $\pm$  SE measured from three independent experiments but the difference on growing rate was not statistically significant.

In agreement with our result, Boyle et al. reported that melanoma cell lines and metastatic melanomas expressed larger amounts of GDF-15 than melanocytes, nevi, and primary lesions of melanoma. Knockdown of GDF-15 expression in three melanoma cell strains tested resulted in a significant decrease in tumorigenicity but did not affect anchorage-independent growth of the cells. The authors demonstrated that, in melanoma cells, expression of GDF-15 was at least partially dependent on the mitogen-activated protein kinase (MAPK) pathway and that stem cell factor-mediated c-Kit activation enhanced the level of GDF-15 [Boyle et al., 2009]. The decrease in GDF-15 levels by shRNA constructs reduced melanoma tumorigenesis, but did not alter cultured cell growth, suggesting a unique function other than growth control. GDF-15 may positively affect tumor progression via the Src-dependent transactivation of ErbB family receptors. Any activation of ErbB family tyrosine kinases by GDF-15 was likely to promote the ability of tumor cells to activate oncogenic signaling, most notably signaling of Akt and MAPKs in SK-BR-3 human breast cancer cells [Park et al., 2010].

In contrast, Baek and co-workers described an anti-tumorigenic function of GDF-15 ectopically expressed in the colon carcinoma cell line HCT-116 [Baek et al., 2001]. Ectopic expression of GDF-15 in the glioblastoma cell line LN-Z308, which are insensitive to GDF-15 mediated growth suppression in vitro, completely abolished tumorigenicity in vivo [Albertoni et al., 2002]. Additionally, GDF-15 expression in MCF-7 cells could directly

inhibit tumor growth in an orthotopic tumor model using MCF-7 cells that overexpress GDF-15 [Martinez et al., 2006].

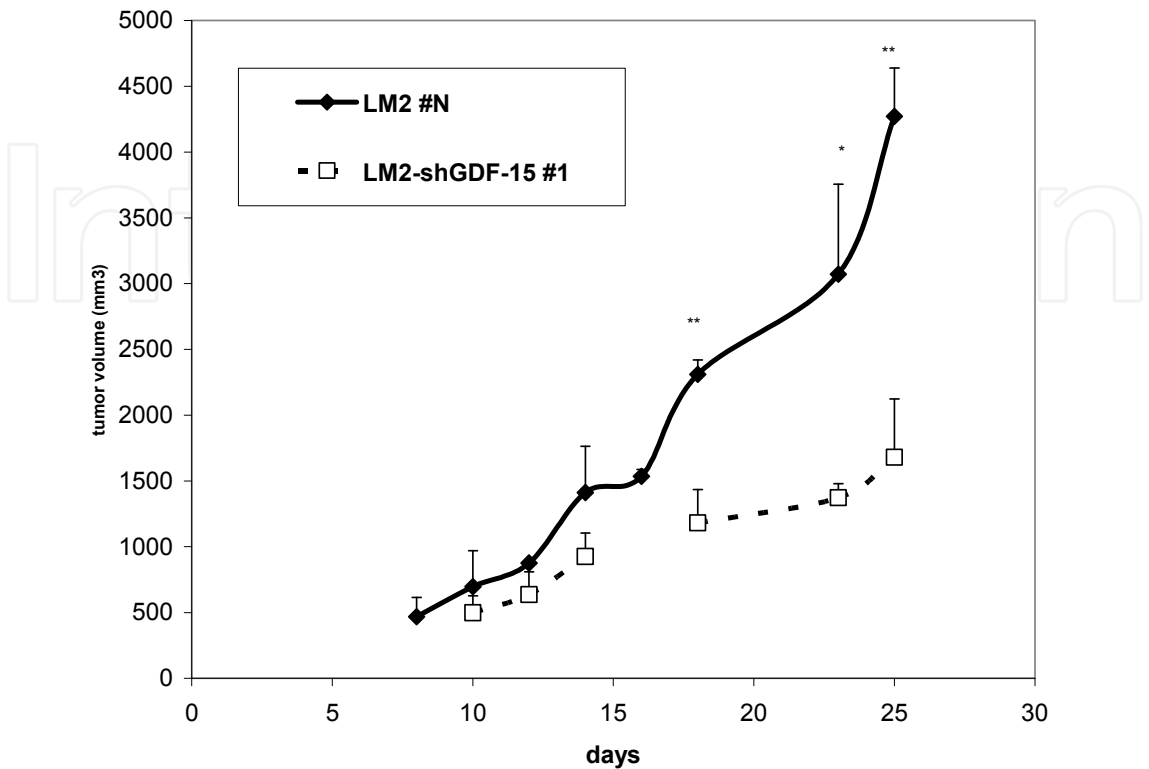


Fig. 6. b Effect of GDF-15 expression on tumor growth in vivo.

For statistical analysis Pearson’s t test were used. Differences were accepted as statistically significant if \*p<0.05 and \*\*p<0,001

We speculated that the inhibitory or tumor promoter effect of GDF-15 on different tumor cell growth might be due to a defect in the TGF-β/Smad signaling pathway or the presence of the wild type p53 mediated cascade.

**3.6 Therapeutic efficiency of combined radiotherapy with stable overexpression of shGDF-15 in preclinical mouse mammary tumor model**

Irradiation was carried out using a cobalt-60 source at a dose rate of 0.45 Gy/ min-, the total given dose was 4 Gy. For the growth delay assay, mice were given local irradiation. The two dimensions of each tumor were measured every second day with digital calipers, and the tumor volume was estimated using the formula  $\Pi/6 \times w1 \times w2^2$  product of the longest (w1) and shortest (w2) dimensions.

To assess the long-term consequences of GDF-15 depletion in LM2 shGDF-15#1 cells on radiosensitivity, 4 Gy local exposure was applied, followed by implantation into Balb/c mice, to monitor the tumor growth. Cells depleted of GDF-15 showed no difference in tumor-forming capacity compared with LM2#N control cells (Figure 7), consistently with the modest in vitro effect. However, significant radiation-delayed tumor growth was observed compared with unirradiated LM2-shGDF-15#1 cells. The cytotoxic effect of 4 Gy exposure was significantly enhanced in GDF-15 depleted LM2 cells (p<0.05 in one-way ANOVA).

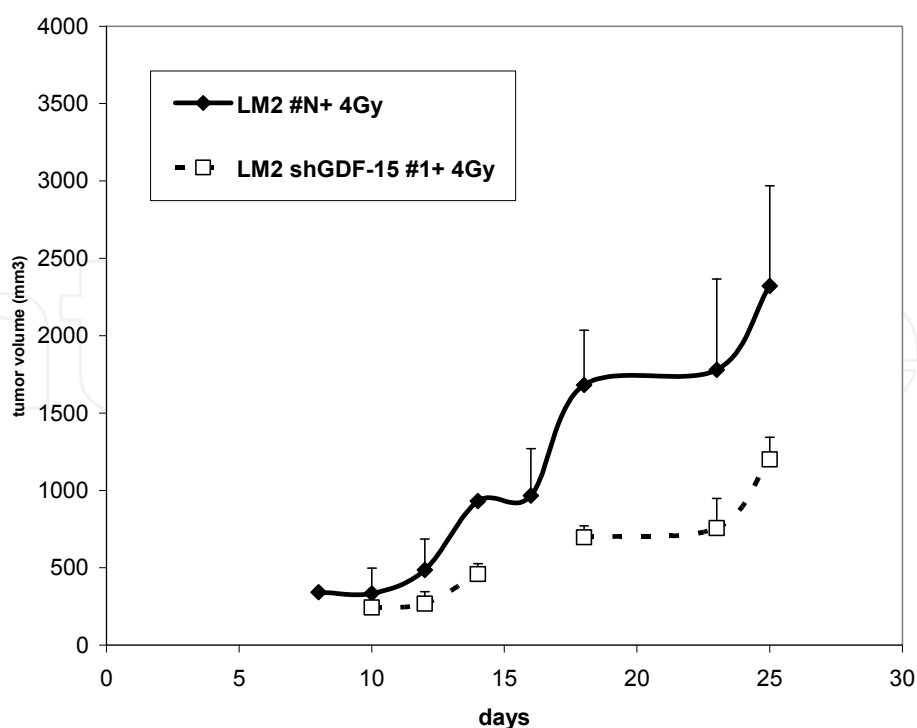


Fig. 7. Enhanced cytotoxicity and antitumor activity of a combination of GDF-15 silencing and ionizing radiation.

LM2 #N or LM2-shGDF-15#1 syngrafts grown in Balb/c mice were treated with ionizing radiation and tumor growth was monitored. A week after LM2 ( $5 \times 10^5$  cell/50  $\mu$ l) cell injection, animals were given radiation treatment (4 Gy).

#### 4. Conclusion

In order to investigate the role of GDF-15 in the cellular response to ionizing radiation, a cell line in which GDF-15 expression was silenced by shRNA interference was generated. In summary, in the present study we were able to demonstrate that GDF-15 silencing combined with irradiation has additive effects on clonogenic survival in-vitro, and the tumor growth delay in vivo. Furthermore, we showed that silencing of GDF-15 with shRNA causes radiosensitization of LM2 cells. This suggests that GDF-15 is an attractive target to improve the efficacy of radiotherapy. Additional radiobiological studies are necessary to investigate the role of GDF-15 and its association with radiosensitivity of other tumor cell lines.

Based on careful dissection of the complicated series of signaling changes within multiple pathways, it may in the future be possible to rationally combine multiple inhibitors of these processes to block cell survival, including inhibition of DNA damage sensing, receptor activation, paracrine ligand evolution, and intracellular signaling pathway, to achieve a better therapeutic response to radiotherapy.

#### 5. Acknowledgment

This work was supported by the following grants: the European Union NOTE project (FP6-036465/2006), Hungarian OTKA K77766 and ETT 827-1/2009. The authors appreciate the expert technical assistance of Ms. Mária Frigyesi and Ms. Rita Lőkös.



## 6. References

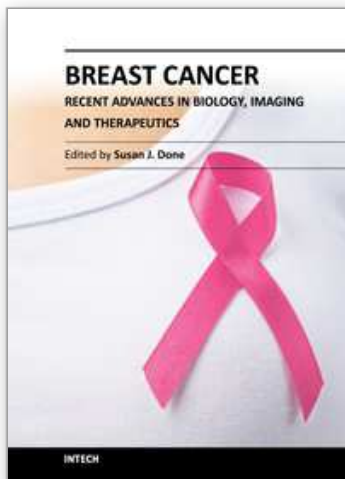
- Albertoni, M., Shaw, P.H., Nozaki, M., Godard, S., Tenan, M., Hamou, M.F., Fairlie, D.W., Breit, S.N., Paralkar, V.M., de Tribolet, N., van Meir, E.G., & Hegi, M.E. (2002). Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. *Oncogene*, Vol. 21, No. 27, 4212–4219.
- Amundson, S.A., Lee, R.A., Koch-Paiz, C.A., Bittner, M.L., Meltzer, P., Trent, J.M. and Fornace, A.J. Jr. (2003). Differential responses of stress genes to low dose-rate  $\gamma$  irradiation. *Mol Cancer Res*, Vol. 1, No. 6, 445–452.
- Anscher, M.S., Peters, W.P., Reisenbichler, H., Petros, W.P., & Jirtle, R.L. (1993). Transforming growth factor beta as a predictor of liver and lung fibrosis after autologous bone marrow transplantation for advanced breast cancer. *N Engl J Med*, Vol. 328, No. 22, 1592–1598.
- Balaban, N., Moni, J., Shannon, M., Dang, L., Murphy, E., & Goldkorn, T. (1996). The effect of ionizing radiation on signal transduction: antibodies to EGF receptor sensitize A431 cells to radiation. *Biochimica et Biophysica Acta-Molecular Cell Research*, Vol. 1314, No. 1-2, 147–156.
- Baek, S.J., Horowitz, J.M. & Eling, T.E. (2001). Molecular cloning and characterization of human nonsteroidal anti-inflammatory drug-activated gene promoter. Basal transcription is mediated by Sp1 and Sp3. *J Biol Chem*, Vol. 276, No. 36, (Jul. 2001), 33384–33392.
- Baek, S.J., Kim, J.S., Jackson, F.R., Eling, T.E., McEntee, M.F., & Lee, S.H. (2004). Epicatechin gallate-induced expression of NAG-1 is associated with growth inhibition and apoptosis in colon cancer cells. *Carcinogenesis*, Vol. 25, No. 12, (Aug. 2004), 2425–2432.
- Baek, S.J., Kim, J.S., Moore, S.M., Lee, S.H., Martinez, J., & Eling, T.E. (2005). Cyclooxygenase inhibitors induce the expression of the tumor suppressor gene EGR-1, which results in the up-regulation of NAG-1, an antitumorigenic protein. *Mol Pharmacol*, Vol. 67, No. 2, (Oct. 2004), 356–364.
- Baek, K.E., Yoon, S.R., Kim, J.T., Kim, K.S., Kang, S.H., Yang, Y., Lim, J.S., Choi, I., Nam, M.S., Yoon, M., Lee, H.G. (2009). Upregulation and secretion of macrophage inhibitory cytokine-1 (MIC-1) in gastric cancers. *Clin Chim Acta*, Vol. 401, No. 1-2, (Dec. 2008), 128–133.
- Bauskin, A.R., Brown, D.A., Junankar, S., Rasiah, K.K., Eggleton, S., Hunter, M., Liu, T., Smith, D., Kuffner, T., Pankhurst, G.J., Johnen, H., Russell, P.J., Barret, W., Stricker, P.D., Grygiel, J.J., Kench, J.G., Henshall, S.M., Sutherland, R.L., & Breit, S.N. (2005). The propeptide mediates formation of stromal stores of PROMIC-1: role in determining prostate cancer outcome. *Cancer Res*, Vol. 65, No. 6, 2330–2336.
- Bauskin, A.R., Brown, D.A., Kuffner, T., Johnen, H., Luo, X.W., Hunter, M., & Breit, S.N. (2006). Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. *Cancer Res*, Vol. 66, No. 10, 4983–4986.
- Boyle, G.M., Pedley, J., Martyn, A. C., Banducci, K.J., Strutton, G.M., Brown, D.A., Breit, S.N., & Parsons, P.G. (2009). Macrophage inhibitory cytokine-1 is overexpressed in malignant melanoma and is associated with tumorigenicity. *Journ Invest Dermatol*, Vol. 129, No. 2, (Aug. 2008), 383–391.
- Brown, D.A., Ward, R.L., Buckhaults, P., Liu, T., Romans, K.E., Hawkins, N.J., Bauskin, A.R., Kinzler, K.W., Vogelstein, B., & Breit, S.N. (2003). MIC-1 serum level and genotype: associations with progress and prognosis of colorectal carcinoma. *Clin Cancer Res*, Vol. 9, No. 7, 2642–2650.

- Buckhaults, P., Rago, C., St Croix, B., Romans, K.E., Saha, S., Zhang, L., Vogelstein, B., & Kinzler, K.W. (2001). Secreted and cell surface genes expressed in benign and malignant colorectal tumors. *Cancer Res*, Vol. 61, No. 19, 6996–7001.
- Calaf, G.M., & Hei, T.K. (2004). Ionizing radiation induces alterations in cellular proliferation and c-myc, c-jun and c-fos protein expression in breast epithelial cells. *Int J Oncol*, Vol. 25, No. 6, 1859-1866.
- Cekanova, M., Lee, S.H., Donnell, R.L., Sukhthankar, M., Eling, T.E., Fischer, S.M., & Baek, S.J. (2009). Nonsteroidal anti-inflammatory drug-activated gene-1 expression inhibits urethane-induced pulmonary tumorigenesis in transgenic mice. *Cancer Prev Res*, Vol. 2, No. 5, (Apr. 2009), 450-458.
- Chaudhry, M.A., Chodosh, L.A., McKenna, W.G., & Muschel, R.J. (2003). Gene expression profile of human cells irradiated in G1 and G2 phases of cell cycle. *Cancer Lett*, Vol. 195, No. 2, 221-233.
- Chen, S.J., Karan, D., Johansson, S.L., Lin, F.F., Zeckser, J., Singh, A.P., Batra, S.K., & Lin, M.F. (2007). Prostate-derived factor as a paracrine and autocrine factor for the proliferation of androgen receptor-positive human prostate cancer cells. *Prostate*, Vol. 67, No. 5, 557-571.
- Chen, Y., Williams, J., Ding, I., Hernady, E., Liu, W., Smudzin, T., Finkelstein, J.N., Rubin P., & Okunieff, P. (2002). Radiation pneumonitis and early circulatory cytokine markers. *Semin Radiat Oncol*, Vol. 12, No. 1, 26–33.
- Contente, A., Dittmer, A., Koch, M.C., Roth, J., & Dobbstein, M. (2002). A polymorphic microsatellite that mediates induction of PIG3 by p53. *Nat Genet*, Vol. 30, No. 3, (feb. 2002), 315-320.
- Cucinotta, F.A., Dicello, J.F., Nikjoo, H., & Cherubini, R. (2002). Computational model of the modulation of gene expression following DNA damage. *Radiat Prot Dosimetry*, Vol. 99, No. 1-4, 85-90.
- Ding, L.H., Shingyoji, M., Chen, F., Hwang, J.J., Burma, S., Lee, C., Cheng, J.F., & Chen, D.J. (2005). Gene expression profiles of normal human fibroblasts after exposure to ionizing radiation: a comparative study of low and high doses. *Radiat Res*, Vol. 164, No. 1, 17-26.
- Dumont, N., & Arteaga, C.L. (2003). Targeting the TGF beta signaling network in human neoplasia. *Cancer cell*, Vol. 3, No. 6, 531–536.
- Galli, S., Colombo, L., Vanzuli, S., Daroqui, M.C., del Carmen Vidal, M., Jasniz, M.A., Sacerdote de Lustig, E., & Eijan, A.M. (2000). Characterization of a fibroblastoid mammary carcinoma cell line (LM2) originated from a mouse adenocarcinoma. *Int J Oncol*, Vol. 17, No. 6, 1259-1265.
- Goldkorn, T., Balaban, N., Shannon, M., & Matsukuma, K. (1997). EGF receptor phosphorylation is affected by ionizing radiation. *Biochimica et Biophysica Acta-Molecular Cell Research*, Vol. 1358, No. 3, 289-99.
- Hegyesi, H., Colombo, L., Pállinger, E., Tóth, S., Boer, K., Molnár, V., Falus, A. (2007). Impact of systemic histamine deficiency on the crosstalk between mammary adenocarcinoma and T cells. *J Pharmacol Sci*, Vol. 105, No. 1, 66-73.
- Helland, A., Johnsen, H., Frøyland, C., Landmark, H.B., Saetersdal, A.B., Holmen, M.M., Gjertsen, T., Nesland, J.M., Ottestad, W., Jeffrey, S.S., Ottestad, L.O., Rodningen, O.K., Sherlock, G., & Børresen-Dale, A.L. (2006). Radiation-induced effects on gene expression: an in vivo study on breast cancer. *Radiother Oncol*, Vol. 80, No. 2, (Aug. 2006), 230-235.
- Hsiao, E.C., Koniaris, L.G., Zimmers-Koniaris, T., Sebald, S.M., Huynh, T.V., & Lee, S.J. (2000). Characterization of growth-differentiation factor 15, a transforming growth

- factor beta superfamily member induced following liver injury. *Mol. Cell Biol*, Vol. 20, No. 10, 3742–3751.
- Huh, S.J., Chung, C.Y., Sharma, A., & Robertson, G.P. (2010). Macrophage inhibitory cytokine-1 regulates melanoma vascular development. *Am J Pathol*, Vol. 176, No. 6, (Apr. 2010), 2948–2957.
- Jutooru, I., Chadalapaka, G., Chintharlapalli, S., Papineni, S., & Safe, S. (2009). Induction of apoptosis and nonsteroidal anti-inflammatory drug-activated gene 1 in pancreatic cancer cells by a glycyrrhetic acid derivative. *Mol Carcinog*, Vol. 48, No. 8, 692–702.
- Kim, K.K., Lee, J.J., Yang, Y., You, K.H., & Lee, J.H. (2008). Macrophage inhibitory cytokine-1 activates AKT and ERK-1/2 via the transactivation of ErbB2 in human breast and gastric cancer cells. (2008). *Carcinogenesis*, Vol. 29, No. 4, (Feb. 2008), 704–712.
- Kelly, J.A., Lucia M.S., & Lambert J.R. (2009). p53 controls prostate-derived factor/macrophage inhibitory cytokine/NSAID-activated gene expression in response to cell density, DNA damage and hypoxia through diverse mechanisms. *Cancer Lett*, Vol. 277, No. 1, (Dec. 2009), 38–47.
- Klos, K.S., Wyszomierski, S.L., Sun, M., Tan, M., Zhou, X., Li, P., Yang, W., Yin, G., Hittelman, W.N., & Yu, D. (2006). ErbB2 increases vascular endothelial growth factor protein synthesis via activation of mammalian target of rapamycin/p70S6K leading to increased angiogenesis and spontaneous metastasis of human breast cancer cells. *Cancer Res*, Vol. 66, No. 4, 2028–2037.
- Koopmann, J., Buckhaults, P., Brown, D.A., Zahurak, M.L., Sato, N., Fukushima, N., Sokoll, L.J., Chan, D.W., Yeo, C.J., Hruban, R.H., Breit, S.N., Kinzler, K.W., Vogelstein, B., & Goggins, M. (2004). Serum macrophage inhibitory cytokine1 as a marker of pancreatic and other periampullary cancers. *Clin Cancer Res*, Vol. 10, No. 7, 2386–2392.
- Lambert, J.R., Kelly, J.A., Shim, M., Huffer, W.E., Nordeen, S.K., Baek, S.J., Eling, T.E., Lucia, M.S. (2006). Prostate derived factor in human prostate cancer cells: gene induction by vitamin D via a p53-dependent mechanism and inhibition of prostate cancer cell growth. *J Cell Physiol*, Vol. 208, No. 3, (sep 2006) 566–74.
- Lee, D.H., Yang, Y., Lee, S.J., Kim, K.Y., Koo, T.H., Shin, S.M., Song, K.S., Lee, Y.H., Kim, Y.J., Lee, J.J., Choi, I., & Lee, J.H. (2003). Macrophage inhibitory cytokine-1 induces the invasiveness of gastric cancer cells by up-regulating the urokinase-type plasminogen activator system. *Cancer Res*, Vol. 63, No. 15, 4648–4655.
- Li, P.X., Wong, J., Ayed, A., Ngo, D., Brade, A.M., Arrowsmith, C., Austin, R.C., & Klamut, H.J. (2000). Placental transforming growth factor-beta is a downstream mediator of the growth arrest and apoptotic response of tumor cells to DNA damage and p53 overexpression. *J Biol Chem*, Vol. 275, No. 26, 20127–20135.
- Li, Y.M., Zhou, B.P., Deng, J., Pan, Y., Hay, N., & Hung, M.C. (2005). A hypoxia-independent hypoxia-inducible factor-1 activation pathway induced by phosphatidylinositol-3 kinase/Akt in HER2 overexpressing cells. *Cancer Res*, Vol. 65, No. 8, 3257–3263.
- Liu, T., Bauskin, A.R., Zaunders, J., Brown, D.A., Pankhurst, S., Russell, P.J., & Breit, S.N. (2003). Macrophage inhibitory cytokine 1 reduces cell adhesion and induces apoptosis in prostate cancer cells. *Cancer Res*, Vol. 63, No. 16, 5034–5040.
- Martinez, J.M., Sali, T., Okazaki, R., Anna, C., Hollingshead, M., Hose, C., Monks, A., Walker, N.J., Baek, S.J., & Eling, T.E. (2006). Drug-induced expression of nonsteroidal anti-inflammatory drug-activated gene/macrophage inhibitory cytokine-1/ prostate-derived factor, a putative tumor suppressor, inhibits tumor growth. *J Pharmacol Exp Ther*, Vol. 318, No. 2, (May. 2006), 899–906.

- Mercier, G., Berthault, N., Mary, J., Peyre, J., Antoniadis, A., Comet, J.-P., Cornuejols, A., Froidevaux, C., & Dutreix, M. (2004). Biological detection of low radiation doses by combining results of two microarray analysis methods. *Nucleic Acids Res*, Vol. 32, No. 1, e12.
- Newman, D., Sakaue, M., Koo, J.S., Kim, K.S., Baek, S.J., Eling, T., & Jetten, A.M. (2003). Differential regulation of nonsteroidal anti-inflammatory drug-activated gene in normal human tracheobronchial epithelial and lung carcinoma cells by retinoids. *Mol Pharmacol*, Vol. 63, No. 3, 557-564.
- Polyak, K., Xia, Y., Zweier, J.L., Kinzler, K.W., & Vogelstein, B. (1997). A model for p53-induced apoptosis. *Nature*, Vol. 389, No. 6648, 300-305.
- Rohde, M., Daugaard, M., Jensen, M.H., Helin K., Nylandsted J., & Jäättelä M. (2005). Members of the heat-shock protein 70 family promote cancer cell growth by distinct mechanisms. *Genes Dev*, Vol. 19, No. 5, 570-582.
- Roy, D., Calaf, G. & Hei, T.K. (2001). Profiling of differentially expressed genes induced by high linear energy transfer radiation in breast epithelial cells. *Mol Carcinog*, Vol. 31, No. 4, 192-203.
- Rubin, P., Finkelstein, J.N., Siemann, D.W., Shapiro, D.L., van Houtte, P., & Penney, D.P. (1986). Predictive biochemical assays for late radiation effects. *Int J Radiat Oncol Biol Phys*, Vol. 12, No. 4, 469-476.
- Senapati, S., Rachagani, S., Chaudhary, K., Johansson, S.L., Singh, R.K., & Batra, S.K. (2010). Overexpression of macrophage inhibitory cytokine-1 induces metastasis of human prostate cancer cells through the FAK-RhoA signaling pathway. *Oncogene*, Vol. 29, No. 9, (Nov. 2009), 1293-1302.
- Selander, K.S., Brown, D.A., Sequeiros, G.B., Hunter, M., Desmond, R., Parpala, T., Risteli, J., Breit, S.N., Jukkola-Vuorinen, A. (2007), Serum macrophage inhibitory cytokine-1 concentrations correlate with the presence of prostate cancer bone metastases. *Cancer Epidemiol Biomarkers Prev*, Vol. 16, No.3, 532-537.
- Tan, M., Wang, Y., Guan, K., & Sun, Y. (2000). PTGF-beta, a type beta transforming growth factor (TGF-beta) superfamily member, is a p53 target gene that inhibits tumor cell growth via TGF-beta signaling pathway. *Proc Natl Acad Sci USA*, Vol. 97, No. 1, 109-114.
- Vallat, L., Magdelénat, H., Merle-Béral, H., Masdehors, P., de Montalk, G.P., Davi, F., Kruhoffer, M., Sabatier, L., Orntoft, T.F., & Delic, J. (2003). The resistance of B-CLL cells to DNA damage induced apoptosis defined by DNA microarrays. *Blood*, Vol. 101, No. 11, (Feb. 2003), 4598-4606.
- Welsh, J.B., Sapinoso, L.M., Kern, S.G., Brown, D.A., Liu, T., Bauskin, A.R., Ward, R.L., Hawkins, N.J., Quinn, D.I., Russell, P.J., Sutherland, R.L., Breit, S.N., Moskaluk, C.A., Frierson, H.F. Jr., & Hampton, G.M. (2003). Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. *Proc Natl Acad Sci USA*, Vol. 100, No. 6, (Mar. 2003), 3410-3415.
- Wilson, L.C., Baek, S.J., Call, A., & Eling, T.E. (2003). Nonsteroidal anti-inflammatory drug-activated gene (NAG-1) is induced by genistein through the expression of p53 in colorectal cancer cells. *Intl. J. Cancer*, Vol. 105, No. 6, 747-753.
- Yamaguchi, K., Lee, S. H., Eling, T. E., & Baek, S.J. (2006). A novel peroxisome proliferator-activated receptor gamma ligand, MCC-555, induces apoptosis via posttranscriptional regulation of NAG-1 in colorectal cancer cells. *Mol Cancer Ther*, Vol. 5, No. 5, 1352-1361.
- Yun Jung Park, Y.J., Lee H., & Lee, H.-J. (2010). Macrophage inhibitory cytokine-1 transactivates ErbB family receptors via the activation of Src in SK-BR-3 human breast cancer cells. *BMB Rep*, Vol. 43, No. 2, 91-96.





## **Breast Cancer - Recent Advances in Biology, Imaging and Therapeutics**

Edited by Dr. Susan Done

ISBN 978-953-307-730-7

Hard cover, 428 pages

**Publisher** InTech

**Published online** 14, December, 2011

**Published in print edition** December, 2011

In recent years it has become clear that breast cancer is not a single disease but rather that the term encompasses a number of molecularly distinct tumors arising from the epithelial cells of the breast. There is an urgent need to better understand these distinct subtypes and develop tailored diagnostic approaches and treatments appropriate to each. This book considers breast cancer from many novel and exciting perspectives. New insights into the basic biology of breast cancer are discussed together with high throughput approaches to molecular profiling. Innovative strategies for diagnosis and imaging are presented as well as emerging perspectives on breast cancer treatment. Each of the topics in this volume is addressed by respected experts in their fields and it is hoped that readers will be stimulated and challenged by the contents.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Hargita Hegyesi, James R. Lambert, Nikolett Sándor, Boglárka Schilling-Tóth and Géza Sáfrány (2011). Validation of Growth Differentiation Factor (GDF-15) as a Radiation Response Gene and Radiosensitizing Target in Mammary Adenocarcinoma Model, Breast Cancer - Recent Advances in Biology, Imaging and Therapeutics, Dr. Susan Done (Ed.), ISBN: 978-953-307-730-7, InTech, Available from: <http://www.intechopen.com/books/breast-cancer-recent-advances-in-biology-imaging-and-therapeutics/validation-of-growth-differentiation-factor-gdf-15-as-a-radiation-response-gene-and-radiosensitizing>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen